Speaker: Carlos Alvarez Lucas, M.O., MSHI
Arturo Andres Sil Plata, M.D., Ph.D.

Topic: NTDs in Mexico: Beyond the Border

Objectives:
1) Describe the magnitude and patterns of NTDs in Mexico.
2) Compare the prevalence of NTDs in border vs. non-border areas of Mexico.
3) Describe 2 areas in which NTD rates are very high.
4) Describe 2 risk factors identified for NTDs in Mexico.
5) Describe nation- or region-wide NTD prevention efforts.

Abstract: The Epidemiological Surveillance System for Neural Tube Defects (NTD) implemented since 1993 by border cities, as well as nationwide, provides information on the cases of anencephaly, spina bifida and cephalocele.

In 1998, Mexico reported an incidence rate of NTD of 8.5 cases per 10,000 registered live births. Sonora, Tamaulipas and Baja California reported incidence rates of 3.9, 6.3 and 6.4 cases per 10,000 registered live births, respectively. Also, two other states had high rates: Puebla (27.7) and the state of Mexico (8.3). Anencephaly is the principal defect with a rate of 6 cases per l0,000 registered live births. The NTD epidemiological information from 1996 to 1999 shows a descending trend.

The analysis of risk factors for the 372 cases reported by the states of Aguascalientes, Baja California, Coahuila, State of Mexico, Puebla and Veracruz, shows a higher risk of anencephaly when the father is a farmer, with an odds ratio (OR) of 6.43 with a 95% confidence interval (CI) of 3.89-10.68, as well as a higher risk of spina bifida, with an OR of 4.12, and CI of 2.18-7.73. In pregnant women with previous NTD, no higher risk of the same problem is observed. The ratio of women to men is 1.8:1.

All the states have strengthened the epidemiological surveillance activities and only some states carry out prevention and control measures.

Speaker: Irina Cech, Ph.D.

Topic: NTD Study on the Texas-Mexico Border

Objectives:
1) Identify the objectives of this study.
2) Describe the participation of Mexican subjects.
3) Summarize study findings.

Abstract Irina Cech, Ph.D.,* Carrie Shapiro,* R.N., M.P.H., Anne Sweeney, Ph.D.,* Bertram W. Roberts, M.D., M.P.H.,* Ines Anchondo, R.N, M.P.H.,* Janet Englund, M.D.,** and Frederick E. Harlass, M.D.*** This project is investigating the interaction of chemical and biological risk factors in the etiology of neural tube defect (NTD) births near the Texas-Mexico border. The study is sponsored by the Agency for Toxic Substances and Disease Registry and covers the entire 14-county Texas border area and Mexican border towns. We hypothesize that maternal infection and exposure to toxic chemicals, solely or in tandem, increase the risk of NTDs, and that maternal diabetes and nutritional deficiencies are possible co-factors. The focus on the *compounded* effects of chemical and biological exposures is a unique contribution this study will make toward a better understanding the etiology of NTD births.

In collaboration with the Texas Department of Health, cases of NTDs (anencephaly and open spina bifida) are ascertained and enrolled at the time of prenatal diagnosis at participating clinics and at delivery in border area hospitals. We enroll four controls to individually match each case on geographic location, maternal age, ethnicity, gestational age at enrollment of the case, and type of insurance. Maternal biological specimens (blood, sera, urine, and hair samples) are obtained at the time of diagnosis for cases and at corresponding gestational ages for controls. Cord blood is collected at delivery or termination.

Biological specimens are analyzed for antibodies to selected pathogens (human parvovirus B19, Coxsackie B viruses, and *Naegleria fowleri* amoeba), as well as for metals (arsenic, lead, inorganic and organic mercury), PCBs, persistent and non-persistent pesticides, and serum folate/vitamin B12. Blood, sera, and urine samples are analyzed by the CDC Laboratory and the Virus Reference Laboratory. Hair samples are analyzed for metals at the University of Texas School of Public Health laboratory.

In parallel with collection of biological specimens, we also collect samples of drinking water, soil, soil gas, and indoor air from maternal residence at conception. For environmental sampling, we travel to the border, and even across the border, if a mother lived in Mexico early in pregnancy. Since the critical time in the NTD pregnancy is the first 28 days of gestation, the study protocol stresses the necessity of accurately reconstructing residential history in order to address chemical and biological exposures appropriately.

The environmental samples are analyzed for fecal indicator bacteria, as well as for a panel of volatile and semi-volatile organic chemicals, pesticides, PCB, nitrates, and uranium decay products. All environmental analytical work is done at the University of Texas School of Public Health laboratories. Additional information is obtained via a survey questionnaire, addressing possible geographic, demographic, environmental, occupational, and genetic factors that may have contributed to NTDs.

A total of 288 participants (95 women with anencephalic or open spina bifida pregnancies and 193 individually matched controls) have been enrolled to date. Of the total, 148 participants, 34 cases and 114 controls, were enrolled during 1999. One hundred and sixty six (166) conception addresses have been visited with environmental sampling, including 21 residences located on the Mexico side. This represents one of the largest environmental quality and health data bases ever collected in the U.S.-Mexico border region. The project is expected to continue until October 2000.

* University of Texas Houston School of Public Heath.
** University of Chicago, Medical School. *** Texas Tech University Medical School.

Speaker: Hope Northrup, M.D. **Topic:** The Search for Spina Bifida Genes

Objectives:

1) State the percentage of spina bifida cases thought to be attributable to genetic causes.
2) Identify one gene alteration that has been implicated in causing neural tube defects.
3) Summarize methods and one significant finding from recent research.

Abstract: Neural tube defects (NTDs) are one of the most common crippling birth defects with an overall incidence in the United States of 1-2 per 1,000 births. NTDs include all congenital anomalies involving lack of closure of the developing neural tube during embryogenesis. Roughly half of NTDs result in meningomyelocele (an exposed area of the spinal cord), which is compatible with life but usually (99% of the time) results in handicap. NTDs are known to occur secondary to a combination of genetic and environmental factors. Our study will identify genes that contribute to the occurrence of meningomyelocele [also termed spina bifida (SB)]. The search for SB-causing genes is complicated by: the lack of clear-cut inheritance in SB, paucity of multigenerational SB families, and the unknown contribution of the phenotype from environmental factors. Our subject population is large (500), simplex SB families (families with a single affected individual) who will be tested for genetic associations. A nonparametric linkage technique, the transmission disequilibrium test (TDT) will be our primary method of analysis. We are testing approximately 100 candidate genes in five categories: genes involved in folate metabolism, genes implicated from animal models, HOX and PAX genes, growth factor and growth factor receptor genes and proto-oncogenes. The data will be analyzed by ethnicity and level of defect.

The Search for Spina Bifida Genes

Hope Northrup, M.D.

Spina Bifida "split spine"

- Neural tube defect (NTD): congenital defect of the central nervous system involving exposed nervous tissue
- Failure of the neural tube to close during the 4th week of pregnancy
- Most common severely disabling birth defect in the United States
- Occurs in 1 of every 2,000 live births in the U.S.

Associated Anomalies

- Hydrocephalus/Arnold Chiari type II
- Other vertebral anomalies
- Neurogenic bladder
- Scoliosis/Kyphosis
- Club foot
- Dislocated/subluxed hips
- Learning disabilities

Heterogeneity in Location of SB

- 85% distal thoracic, lumbar or sacral
- 10% thoracic
- 5% cervical
- Variation among ethnic groups
- Females – more likely to have thoracic and cervical defects
- Males – more likely to have defects affecting lower spine

Incidence

- 1:2,000 in the United States
- 1,500 infants born each year with SB in the U.S.
- Ethnic and geographical variation

Environmental Factors

- Variation in geographic incidence
    - More SB infants born in eastern and southern states
- Maternal glucose metabolism
    - Obese and diabetic women have an increased risk for having a child with a NTD
- Maternal diet
    - Folic acid supplementation before and during early pregnancy reduces risk of NTDs

Genetic Factors

- Family history
  - Parents with one SB child have an increased risk for having an additional child with SB
- Variation in incidence along ethnic and racial lines
  - Hispanic > Caucasian > African-American > Asian-American
- Animal models
  - NTD malformations detected in several mouse models
- Heritability estimated to be 60%

Factors Complicating Search for SB Genes

- Lack of clear-cut inheritance
- Paucity of multigenerational families
- Unknown contribution to the phenotype from environmental factors

Data/Subject Analysis

- Simplex Families
  - Patients, Mothers, Fathers
- Ethnicity
  - Hispanics
  - Non-Hispanic Whites
- Level of Defect
  - Upper (1)
  - Lower (5)

Revised Study Population:

	Hispanic	Caucasian	African-American
Initial Testing	300	200	
Validation	250		30

Categories of Candidate Genes

- Metabolic pathways – folate and glucose
- Mouse models
- HOX and PAX genes
- Growth factor and growth factor receptors
- Proto-oncogenes

Folate Metabolism

- Folic acid supplementation reduces risk of NTDs
- Folate deficiency associated with closure site 1, 2 and 4 defects (Van Allen et al, 1993)
- Folate transported from maternal plasma to neuroepithelial cells of embryonic neural tube by potocytosis

Thermolabile MTHFR C677T

- Reduced MTHFR activity
- Increased enzyme thermolability
- Increased plasma homocysteine
- Decreased plasma folate
- Risk factor for NTDs

MTHFR A1298C

- Reduced MTHFR activity
- Not associated with elevated homocysteine nor decreased folate levels
- Combined heterozygosity for C677T & A1298C results in:
    - lower MTHFR activity
    - elevated homocysteine
    - decreased plasma folate

Data/Subject Analysis

- Homozygous/Combined heterozygous MTHFR mutations
    - Patients, Mothers, Fathers
- Ethnicity
    - Hispanics
    - Non-Hispanic Whites
- Level of defect
    - Upper (1)
    - Lower (5)

Transmission Disequilibrium Test

- Non-parametric linkage method
    - Model-free method of analysis
    - Does not require data on multiple affected family members or unaffected offspring
    - Can be generalized to a marker locus with more than 2 alleles
    - Controls for population stratification
    - Can utilize singe parent-child pairs
- Tests directly for linkage between a disease and marker locus which shows a population association
- Considers parents heterozygous for an allele associated with a disease and evaluates the frequency with which that allele, or its alternate, is transmitted to affected offspring

Speaker: Larry Kramer, M.D.
Jack Fletcher, M.D.

Topic: Brain Malformations, Learning, and Development in Children with Spina Bifida

Abstract: In addition to the spinal lesion, many children with spina bifida meningomyelocele (SBM) have brain malformations. The Arnold-Chiari II malformation of the cerebellum and hindbrain is well-known. This malformation leads to hydrocephalus in many children with SBM. However, many children with SBM have partial agenesis of the corpus callosum. This agenesis is congenital and most apparent at the ends of the corpus callosum. The agenesis is not due to effects of hydrocephalus, which leads to thinning, or hypoplasia of the corpus callosum. Each of these components of the neural phenotype **B** cerebellum, corpus callosum, and hydrocephalus **B** vary across individual children. The variations may be associated with learning strengths and weaknesses in individual children. In this presentation, the neural phenotype and its relationship with the learning characteristics of learning with SBM will be discussed.